December 22, 1999

VETERINARY SERVICES MEMORANDUM NO. 800.89

Subject: Chicken Anemia Virus

To: Biologics Licensees, Permittees, and Applicants
Directors, Center for Veterinary Biologics

I. PURPOSE

This memorandum provides guidance for licensees, permittees, and applicants interested in using chicken anemia virus (CAV) in licensed facilities for product testing or for the preparation of live or killed CAV vaccines according to 9 CFR 103.1 and 102.5.

II. CANCELLATION

This memorandum cancels Veterinary Services Memorandum No. 800.89 dated February 24, 1997.

III. BACKGROUND

Chicken anemia virus is a non-enveloped, single-stranded, circular DNA virus classified in the genus Circovirus (F. Murphy et al., Virus Taxonomy, 1995). It can cause anemia, immunosuppression, and mortality in young chickens. CAV is widespread throughout the U.S. poultry industry and is extremely stable in the environment. The Center for Veterinary Biologics (CVB) is interested in preventing biological product contamination with CAV and encouraging CAV vaccine development.

A. Product Contamination

Title 9 CFR 102.3(b) requires that applicants establish the purity of a biological product prior to issuance of a U.S. Veterinary Biological Product License. However, the tests found in the current Standard Requirements for viral products (9 CFR 113.200 and 113.300) are insufficient to detect CAV contamination. Therefore, the CVB-Laboratory (CVB-L) now performs a polymerase chain reaction (PCR) test for CAV contamination when conducting confirmatory testing of new poultry Master Seed Viruses (MSVs). CVB is also reviewing the purity regulations for poultry products. While this review is not complete, we plan to include CAV testing in a future Proposed Rule; and we will need input from the biologics industry on the most feasible means for such testing. In anticipation of that proposal, we encourage firms to begin CAV testing of all new poultry MSVs to assist in the development of a mutually agreeable Standard Requirement. While it is not mandatory until a Standard Requirement is in place, prospective licensees should test new poultry MSVs for CAV contamination prior to the submission of MSV samples

to CVB-L for confirmatory testing. Furthermore, we encourage firms to develop a program for testing previously approved poultry MSVs for CAV contamination.

B. Vaccine Development.

On November 25, 1991, CVB issued a Veterinary Biologics Notice entitled "Chicken Anemia Virus Vaccines." Because the ecology of the virus was incompletely understood at that time, we indicated that we would consider license applications for killed CAV vaccines. Since then, more has been learned about this virus; and we now accept applications for licensure of live or killed CAV vaccines.

IV. GUIDELINES

A. Poultry MSV Testing

Until a standard assay can be established, CVB suggests the following options for CAV testing of poultry MSVs. Each firm must develop the specifics of these tests. CVB will also consider other test methods developed by individual firms.

1. *PCR Test* - Conduct this test directly on the MSV sample. Upon request, CVB-L can make available the method they use to perform their PCR test. Alternatively, individual firms could develop similar tests[1,2].

2. *Virus Isolation*

a. Firms may evaluate the MSV sample directly for the presence of live CAV by inoculation of MDCC-MSB1 cells. Isolation procedures should consist of 8-12 passages (every 48-72 hours) over a 3-week period. Firms may score cultures as positive or negative based on the presence or absence of cytopathogenic effect (CPE). However, firms should confirm the induction of CPE by CAV on a representative number of cultures using a technique which employs a CAV-specific antibody reagent (e.g., virus neutralization or fluorescent antibody techniques)[3,4,5].

b. Firms should report the results of tests conducted on new MSVs to CVB on the APHIS Form 2008 they submit when requesting confirmatory testing of the MSV. Individual firms should submit the test method used, along with data defining the sensitivity and specificity of the method described, with the data package accompanying the APHIS Form 2008. CVB also encourages firms to test previously approved MSVs for CAV and to immediately report any positive results to CVB-Inspection and Compliance (CVB-IC).

B. Facility Recommendations

Because CAV is environmentally stable, the licensee should make every effort to prevent CAV contamination of biological products within an establishment.

1. *CAV in Laboratory Facilities* - When CAV is present as an agent in a firm's quality assurance testing laboratory, the firm must manage the laboratory facilities, personnel, equipment, supplies, and test animals in such a manner as to prevent contamination of the production facility with this virus.

2. *CAV in Production Facilities*

a. When CAV is present as an MSV in the production facility, the most direct method of preventing product cross-contamination with this virus is for the firm to establish CAV production facilities which are physically separate and apart from the production of all other products. CVB-IC personnel will determine the adequacy of this separation upon review and inspection.

b. If the firm does not establish separate and apart CAV production facilities, then it should identify, evaluate, and minimize all the potential means of facility contamination throughout the production process. In addition, the firm should test all other poultry products prepared in that facility for CAV contamination. Such firms would not need to test killed products, provided they show the inactivation procedure for the killed product is capable of inactivating CAV as described in Section IV. B. of VS Memorandum No. 800.81.

3. *Disinfection* - In any facility (laboratory or production) in which CAV is present, the firm should propose and validate, with appropriate studies, the cleaning and disinfection methods used in the facility[6].

C. CAV Vaccine Recommendations

For licensure, a killed or live CAV vaccine should meet the requirements in 9 CFR 113.200 and 113.300, guidelines provided in CVB General Licensing Considerations Nos. 800.200 and 800.201, and the following specific licensing considerations:

1. *CAV Vaccine Substrates* - Because of the fastidious growth requirements of this virus, CVB will consider any of the documented growth systems (MDCC-MSB1 cell culture, chicken embryos, and chicken tissues, e.g., liver) as potential substrates for a killed or live CAV vaccine. However, firms

should give due consideration to the potential for contamination with extraneous agents inherent to the substrate chosen.

a. Firms should derive embryos or chicks used in production from specific pathogen free (SPF) flocks, as defined in VS Memorandum No. 800.65. Since SPF flocks are not necessarily CAV-free, firms should monitor source flocks to ensure they remain seronegative for antibodies to CAV for 3 weeks following the collection of the eggs or chicks used in vaccine production.

b. Chicks used as a substrate should be reared, inoculated, and harvested in a facility dedicated to this activity. If not, the producer should conduct an additional final product test to demonstrate the absence of concurrent infection of production chickens by other organisms used in the production facility, as described in the supplemental test found in Section V of VS Memorandum No. 800.81.

2. *CAV Vaccine Efficacy* - CAV causes clinical disease only in young chicks. Chicks can become infected either through vertical or horizontal transmission. Therefore, the label claim for any CAV vaccine (live or killed) should be for the prevention of clinical signs in chicks. This claim should be supported by a challenge study conducted on progeny derived from vaccinated dams.

3. *CAV Vaccine Safety* - The principal safety concern, with either a live or a killed CAV vaccine, is the possible transmission of live CAV vertically to the offspring of vaccinated dams.

a. Firms should evaluate live CAV vaccines for their ability to shed from vaccinated hens (both in feces and in eggs) and to spread to contact chickens. Firms should use these data to establish an upper age limit for vaccination so that vaccination does not result in the shedding of vaccine virus in eggs.

b. Killed CAV vaccine production must include an adequate test to demonstrate complete inactivation of the virus.

/s/ Thomas E. Walton for

Alfonso Torres
Deputy Administrator
Veterinary Services

REFERENCES

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[3]Goryo, M., T. Suwa, S. Matsumoto, T. Umemura, and C. Itakura. Serial propagation and purification of chicken anemia agent in MDCC-MSB1 cell line. Avian Pathol. 16:149-163. 1987.

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